FORM PTO-1390 (REV 12-29-99) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
40168	09/530219

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE98/03155	27 October 1998	27 October 1997

TITLE OF INVENTION
INHIBITOR PROTEIN OF THE WNT SIGNAL PATHWAY

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☐ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☑ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☑ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☑ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☑ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☑ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☑ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☑ Other items or information:

    International Search Report

    International Preliminary Examination Report

    Computer Readable Form of Sequence Listing

    Copy of page 1 of the published PCT application

526 Rec'd PCT/PTO 27 APR 2000

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/530219	INTERNATIONAL APPLICATION NO. PCT/DE98/03155	ATTORNEY'S DOCKET NUMBER 40168

17. ☑ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	9 - 20 =		X **$18.00**	$	
Independent claims	1 - 3 =		X **$78.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$260.00**	$ 260.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1.100.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 1,100.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 1,100.00	
				Amount to be refunded:	$
				charged:	$

a. ☑ A check in the amount of $ 1,100.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 18-2220. A duplicate copy of this sheet is enclosed.

The Declaration/Power of Attorney, Assignment and Small Entity Declaration will be filed shortly.

Priority is claimed from DE 197 47 418.7 filed 27 October 1997.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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An Inhibitor Protein of the wnt Signal Path

The present invention relates to an inhibitor protein of the wnt signal path, a DNA encoding such a protein, and a process for preparing such a protein. Furthermore, this invention concerns the use of the DNA and the protein as well as antibodies directed against the protein.

The wnt signal path plays an important part for the regulation of cell proliferation and differentiation during the embryonal development of Drosophila, *Xenopus laevis* and mice. The wnt signal path comprises the combination of secretory glycoproteins encoded by wnt genes, e.g. Xwnt-8, and wnt receptors to which the glycoproteins bind. In addition, the wnt signal path in man is causally implied in the colon and mammary carcinomas as well as the melanomas (cf. Peifer, M., Science 275, (1997), 1752-1753). Therefore, inhibitors of the wnt signal path could represent a possibility of taking therapeutic against tumoral diseases.

Thus, it is the object of the present invention to provide a product by which the wnt signal path can be inhibited.

According to the invention this is achieved by the subject matters defined in the claims.

Therefore, the subject matter of the present invention relates to an inhibitor protein of the wnt signal path, the protein comprising at least one of the amino acid consensus sequences I and II, indicated in fig. 1.

The present invention is based on the applicant's finding that in animals, particularly mammals, very particularly human beings, there is exists a protein which inhibits the wnt signal path. The applicant has found that *in Xenopus laevis* the expression of the wnt gene, Xwnt-8, results in the formation of Siamese twins. This anomaly will be prevented if the above protein is expressed simultaneously. This protein is

a secretory protein of about 40 kD. It has at least one of the amino acid consensus sequences I and II rich in cysteine and indicated in fig. 1. Variants of the protein are indicated in the form of their DNAs in figure 2. The applicant has also found that variants of the protein are expressed in differing tissues (cf. Table 1 and figure 3).

The present invention refers to the above protein as "wnt inhibitor" (wnt-I).

In a preferred embodiment, (wnt-I) has the amino acid consensus sequences I and II indicated in fig. 1.

A further subject matter of the invention relates to a nucleic acid coding for (wnt-I). It can be an RNA or a DNA. The latter may be a genomic DNA or a cDNA, for example. A DNA is preferred which comprises the following:

the DNA of fig. 2 or a DNA differing therefrom by one or several base pairs,

a DNA hybridizing with the DNA of (a), or

a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

The expression "hybridizing DNA" refers to a DNA which hybridizes with a DNA of (a) under normal conditions, particularly at 20°C below the melting point of the DNA.

The DNA of fig. 2 comprises seven DNAs originating from *Xenopus laevis*, mice, human beings or chickens and coding for (wnt-I). Six of these DNAs were deposited with the DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen* [German-type collection of micro-organisms and cell cultures]) on September 19, 1997 as follows:

Fig. 2.1 (DNA from human beings) as phdkk-3 under DSM 11762
Fig. 2.2 (DNA from chickens) is termed pcdkk-3
Fig. 2.3 (DNA from mice) as pmdkk-2 under DSM 11759
Fig. 2.4 (DNA from human beings) as phdkk-2 under DSM 11761
Fig. 2.5 (DNA from mice) as pmdkk-1 under DSM 11758
Fig. 2.6 (DNA from human beings) as phdkk-1 under DSM 11760
Fig. 2.7 (DNA from *Xenopus laevis*) as pRNdkk-1 under DSM 11757

A DNA according to the invention is described below in the form of a cDNA. It is exemplary for every DNA falling under the present invention.

For the preparation of a cDNA according to the invention it is favorable to use a *Xenopus laevis* cDNA library as a basis (cf. Glinka, A. et al., Mechanisms Develope 60, (1996), 221-231). Corresponding mRNAs are synthesized from the individual cDNA clones by means of RNA polymerase. They are microinjected into *Xenopus laevis* together with mRNA of wnt genes, e.g. Xwnt-8. *Xenopus laevis* is screened for the development of Siamese twins. The latter are obtained when the mRNA of the wnt gene is microinjected as such or together with such a *Xenopus laevis* RNA which does not code for (wnt-I). Thus, the non-occurrence of Siamese twins is evaluated as an evidence for the presence of an mRNA coding for (wnt-I). Such an mRNA reveals directly the corresponding cDNA.

A cDNA according to the invention can be present in a vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for E. coli these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, cDM8 and pCEV4 have to be indicated for the expression in animal cells. The baculovirus expression vector pAcSGHisNT-A is especially suitable for the expression in insect cells.

The person skilled in the art knows suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the E. coli strains HB101, DH1, x1776, JM101, JM109, BL21 and SG 13009, the yeast strain *Saccharomyces cerevisiae* and the animal cells L, 3T3, FM3A, CHO, COS, Vero and HeLa as well as the insect cells sf9.

The person skilled in the art knows in which way a cDNA according to the invention has to be inserted in an expression vector. He is also familiar with the fact that this cDNA can be inserted in combination with a DNA coding for another protein and peptide, respectively, so that the cDNA according to the invention can be expressed in the form of a fusion protein.

Furthermore, the person skilled in the art knows conditions of culturing transformed cells and transfected cells, respectively. He is also familiar with processes of isolating and purifying the protein expressed by the cDNA according to the invention. Thus, such a protein, which may also be a fusion protein, also represents a subject matter of the present invention.

A further subject matter of the present invention relates to an antibody directed against an above protein and fusion protein, respectively. Such an antibody can be prepared by common methods. It may be polyclonal and monoclonal, respectively. For its preparation it is favorable to immunize animals - particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above (fusion) protein or with fragments thereof. Further "boosters" of the animals may be effected with the same (fusion) protein or with fragments thereof. The polyclonal antibody may then be obtained from the animal serum and egg yolk, respectively. As regards the monoclonal antibody, animal spleen cells are fused with myeloma cells.

The present invention enables to better investigate and understand the wnt signal path. (wnt-I) can be detected in organisms by an antibody according to the invention. In addition, an autoantibody directed against this protein can be detected by a (wnt-I) according to the invention. Both detections can be made by common methods, particularly a Western blot, an ELISA, an immunoprecipitation or by immunofluorescence. Moreover, the expression of the gene coding for (wnt-I) can be detected by a nucleic acid according to the invention, particularly a DNA and primers derived therefrom. This detection can be made as usual, particularly in a Southern blot.

Thus, the present invention also serves for better investigating, i.e. diagnosing, and understanding processes which are connected with the wnt signal path. These are e.g. cell proliferation and differentiation as well as diseases of the most varying kinds. Examples of the latter are diseases of the eyes and bones as well as tumoral diseases, particularly colon and mammary carcinomas as well as melanomas.

Besides, the present invention is suitable to take measures for and against the presence of (wnt-I) in organisms. (wnt-I) can be inhibited in organisms by means of an antibody according to the invention. On the other hand, the amount of (wnt-I) in organisms can be increased by a (wnt-I) according to the invention, particularly after linkage to a protein which is not considered foreign by the body, e.g. transferrin or BSA. The same can also be achieved correspondingly by means of a nucleic acid according to the invention, particularly a DNA, which is controlled by a promoter inducible in certain tissues and which after its expression results in the provision of (wnt-I) in these tissues. In addition, a nucleic acid according to the invention, particularly a DNA, can also be used to inhibit (wnt-I). For this purpose, the nucleic acid is used e.g. as a basis for preparing anti-sense oligonucleotides for the expression inhibition of the gene

coding for (wnt-I).

Thus, the present invention also provides the possibility of interfering with the wnt signal path in an activating fashion and inhibitory fashion, respectively. The former could be made e.g. by administration of an antibody according to the invention against (wnt-I). For the latter, it is an obvious thing to administer (wnt-I) according to the invention. The activation of the wnt signal path could be useful if it is considered to culture organisms for the purpose of organ donation. However, the inhibition of the wnt signal path offers itself so as to be able to take therapeutic steps in the case of diseases of bones and eyes as well as tumoral diseases, particularly colon and mammary carcinomas as well as melanomas.

In particular, the present invention distinguishes itself in that it can be used in tissue-specific fashion. This applies to both diagnosis and treatment. For example, a DNA according to the invention, Dkk-1, a corresponding protein and an antibody thereof, respectively, are particularly suitable for tissues, such as brain, heart, vessels, bones, cartilage, connective tissue and eye. Furthermore, a DNA according to the invention, Dkk-2, a corresponding protein and antibody thereof, respectively, are particularly suitable for tissues, such as brain, heart, vessels, bones, connective tissue, kidneys, testes, spleen, ovaries, muscles, uteri, cartilage, eyes and mammas. Moreover, a DNA according to the invention, Dkk-3, a corresponding protein and an antibody thereof, respectively, are particularly suitable for tissues, such as brain, heart, vessels, bones, cartilage, eyes, connective tissue, lungs, ovaries, muscles and mammas.

Brief description of the drawings:

Fig. 1 shows the amino acid consensus sequences I and II of a (wnt-I) according to the invention. The indication

"-" stands for an amino acid, the number of amino acids being variable when they are provided with an asterisk,

fig. 2 shows the base sequence of seven DNAs coding for (wnt-I) by indicating the bases contributing to the amino acid consensus sequences of (wnt-I),

fig. 3 shows the expression of three DNAs coding for (wnt-I), Dkk-1, Dkk-2 and Dkk-3, in tissues.

The present invention is explained by the below examples.

Example 1: Preparation and purification of a (wnt-I) according to the invention

For the preparation of a (wnt-I) according to the invention, the DNA of fig. 2.6, phdkk-1, was provided with Bam HI linkers, then cleaved by Bam HI and inserted in the expression vector pQE-8 (Diagen) cleaved by Bam HI. The expression plasmid pQ/wnt-I was obtained. Such a plasmid codes for a fusion protein comprising 6 histidine residues (N terminus partner) and a (wnt-I) according to the invention (C terminus partner). pQ/wnt-I was used for transforming E. coli SG 13009 (cf. Gottesman, S. et al., J. Bacteriol. 148, (1981), 265-273). The bacteria were cultured in an LB broth with 100 µg/ml ampicillin and 25 µg/ml kanamycin, and induced with 60 µM isopropyl-ß-D-thiogalactopyranoside (IPTG) for 4 h. Lysis of the bacteria was achieved by the addition of 6 M guanidine hydrochloride. Thereafter, chromatography (Ni-NTA resin) was carried out with the lysate in the presence of 8 M urea in accordance with the instructions from the manufacturer (Diagen company) of the chromatography material. The bound fusion protein was eluted in a buffer having a pH of 3.5 After its neutralization, the fusion protein was subjected to 18 % SDS polyacrylamide gel electrophoresis and dyed with coomassie blue (cf. Thomas, J.O. and Kornberg, R.D., J. Mol. Biol. 149

(1975), 709-733).

It showed that a (fusion) protein according to the invention can be prepared in highly pure form.

Example 2: Preparation and detection of an antibody according to the invention

A fusion protein of Example 1 according to the invention was subjected to 18 % SDS polyacrylamide gel electrophoresis. After dyeing the gel with 4 M sodium acetate, an about 40 kD band was cut out of the gel and incubated in phosphate-buffered salt solution. Gel pieces were sedimented before the protein concentration of the supernatant was determined by SDS polyacrylamide gel electrophoresis which was followed by coomassie blue staining. Animals were immunized with the gel-purified fusion protein as follows:

Immunization protocol for polyclonal antibodies in rabbits

35 μg of gel-purified fusion protein in 0.7 ml PBS and 0.7 ml of complete Freund's adjuvant and incomplete Freund's adjuvant, respectively, were used per immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
Day 14: 2nd immunization (incomplete Freund's adjuvant; icFA)
Day 28: 3rd immunization (icFA)
Day 56: 4th immunization (icFA)
Day 80: bleeding to death.

The rabbit serum was tested in an immunoblot. For this purpose, a fusion protein of Example 1 according to the invention was subjected to SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (cf. Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10, (1984), 203-209). The Western blot analysis was carried out as

described in Bock, C.-T. et al., Virus Genes 8, (1994), 215-229. For this purpose, the nitrocellulose filter was incubated with a first antibody at 37°C for one hour. This antibody was the rabbit serum (1:10000 in PBS). After several wash steps using PBS, the nitrocellulose filter was incubated with a second antibody. This antibody was an alkaline phosphatase-coupled monoclonal goat anti-rabbit IgG antibody (Dianova company) (1:5000) in PBS. 30 minutes of incubation at 37°C were followed by several wash steps using PBS and then by the alkaline phosphatase detection reaction with developer solution (36 µM 5'-bromo-4-chloro-3-indolyl phosphate, 400 µM nitroblue tetrazolium, 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) at room temperature, until bands were visible.

It showed that polyclonal antibodies according to the invention can be prepared.

Immunization protocol for polyclonal antibodies in chickens

40 µg of gel-purified fusion protein in 0.8 ml PBS and 0.8 ml of complete Freund's adjuvant and incomplete Freund's adjuvant, respectively, were used per immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
Day 50: 3rd immunization (icFA)

Antibodies were extracted from egg yolk and tested in a Western blot. Polyclonal antibodies according to the invention were detected.

Immunization protocol for monoclonal antibodies in mice

12 µg of gel-purified fusion protein in 0.25 ml PBS and 0.25 ml of complete Freund's adjuvant and incomplete Freund's

adjuvant, respectively, were used per immunization. The fusion protein was dissolved in 0.5 ml (without adjuvant) in the 4th immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
Day 56: 3rd immunization (icFA)
Day 84: 4th immunization (PBS)
Day 87: fusion

Supernatants of hybridomas were tested in a Western blot. Monoclonal antibodies according to the invention were detected.

Table 1: Expression of DNAs according to the invention in mouse embryos

	Dkk-1	Dkk-2	Dkk-3
Neuroepithelium			
E9.5 diencephalon	+++ ventral	+++ medial	+ medial
E12.5	telencephalon M/mantle	hypothalamus	telencephalon M/ventricular zone
Eye	pigmented epithelium	choroid	retina
Spinal cord	-/+	-	ventricular zone Roof plate
Mesoderm:			
Heart E10	bulbis cordis Endocardium septum trans-versum	endothelium	myocardium
Heart E12	endocardial cushion	endothelium	endocard. cushion
Blood vessels	+++ aorta	+++ pulmonary artery	+++ aorta + pulmonary artery
Limbbud mesemchyme	E9 S	I	D
Bone E12	perichondrium	S/mesenchyme	perichondrium I/mesenchyme
Bone E15	Ossification centers	-	-
Urogenital	nephric duct S-shaped body Comma shaped body	metanephric mesenchyme	-
Palate	+++	++	+
Hair follicle	+++ mesenchyme + epithelium	+ -	+ -
Tooth mesenchyme	-	-	+++
Trunk mesoderm	+/-	+++	++

Legend: Mesoderm: (D) deep, (I) intermediate, (L) lateral, (M) medial, (S) superficial
Intensity of expression: (-) absence, (+/-) very weak expression, (+) medium, (++) strong (+++) very strong.

Amended Claims

1. An inhibitor protein of the wnt signal path, wherein the protein comprises at least one of the amino acid consensus sequences I and II indicated in fig. 1 and is encoded by the DNA of figure 2.1, 2.3, 2.4, 2.5, 2.6 or 2.7 and by a DNA related with this DNA via the degenerated genetic code, respectively.

2. DNA coding for the protein according to claim 1, wherein the DNA is that of figures 2.1, 2.3, 2.4, 2.5, 2.6 or 2.7 and a DNA related with this DNA via the degenerated genetic code, respectively.

3. An expression plasmid, comprising the DNA according to claim 2.

4. A transformant, containing the expression plasmid according to claim 3.

5. A process for the preparation of the protein according to claim 1, comprising the culturing of the transformant according to claim 4 under suitable conditions.

6. Antibodies directed against the protein according to claim 5.

7. Use of a protein which comprises at least one of the amino acid consensus sequences I and II, indicated in figure 1, and a DNA coding for such a protein, respectively, for interfering diagnostically and/or therapeutically with the wnt signal path.

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Abstract of the Disclosure

The present invention relates to an inhibitor protein of the wnt signal path, a DNA encoding such a protein and a process for the preparation of such a protein. In addition, this invention concerns the use of the DNA and the protein as well as antibodies directed against the protein.

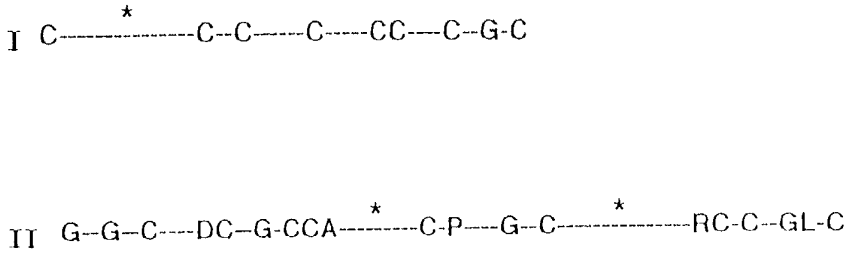

Fig. 1

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  1 ................................................GCGGTGGCGGCC   phdkk-3    2.1
  1 ............................................................   pcdkk-3    2.2
  1 ............................................................   pmdkk-2    2.3
  1 ............................................................   phdkk-2    2.4
  1 ...........................GACCCACGCGTCCGTGCCTGTTTGCGTCCTtCG   pmdkk-1    2.5
  1 ......................................TGGCCCCGCACGCCAAAAATTC   phdkk-1    2.6
  1 GACAGTCGGAGCCGGCGCTGCAGCATCAAAGGGACTTATCTTGGAGGACTTGTGAATTCT   pRNdkk-1   2.7

 13 GCTCTAGAATAGTGGATCCCCCGGGCTGCAGGAATTCGGCACGAGCGGCTGCGGGCGCAG   phdkk-3
  1 .........................................................CGG   pcdkk-3
  1 ............................................................   pmdkk-2
  1 ............................................................   phdkk-2
 34 GAGATGATGGTTGTGTGTGCAcCGGCAGCTGTCCGGTTCTTGGCCGTGTTTACAATGATG   pmdkk-1
 23 GGCACgAGGGTCTgGcactcagagGATGCTCTGACCTTGAAAGGGTCCTATCTGGAGACG   phdkk-1
 61 CATCCTGCCATTGTGGTTACTGAGTCTGGTTGGACAGAGGAATGGGCAGCAACATGTTCC   pRNdkk-1

 73 AGCGGAGATGCAGCGGCTTGGGGCCACCCTGCTGTGCCTGCTGCTGGCGGCGGCGGTCCC   phdkk-3
  4 CGAGCGGCAGCGGCGGCTGAGGAGCGCCGGGGATGCGGCGGGGAGAGGGACCGGCGCCGC   pcdkk-3
  1 ............................................................   pmdkk-2
  1 ............................................................   phdkk-2
 94 GCTCTCTGCAGCCTCCCTCTGCTAGGAGCCAGTGCCACCTTGAACTCAGTTC..TCATCA   pmdkk-1
 83 AGGGAGTACAACGTGCTGAATGTGTGCGGTTCAGGGAGCATTTGGTAACCCT..GCaTTT   phdkk-1
121 CGGTGCCTCTTATTGTCTTTTGGGGTTTTATCTTGGATGGGGCACTTGGCTTTGTCATGA   pRNdkk-1
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Fig. 2

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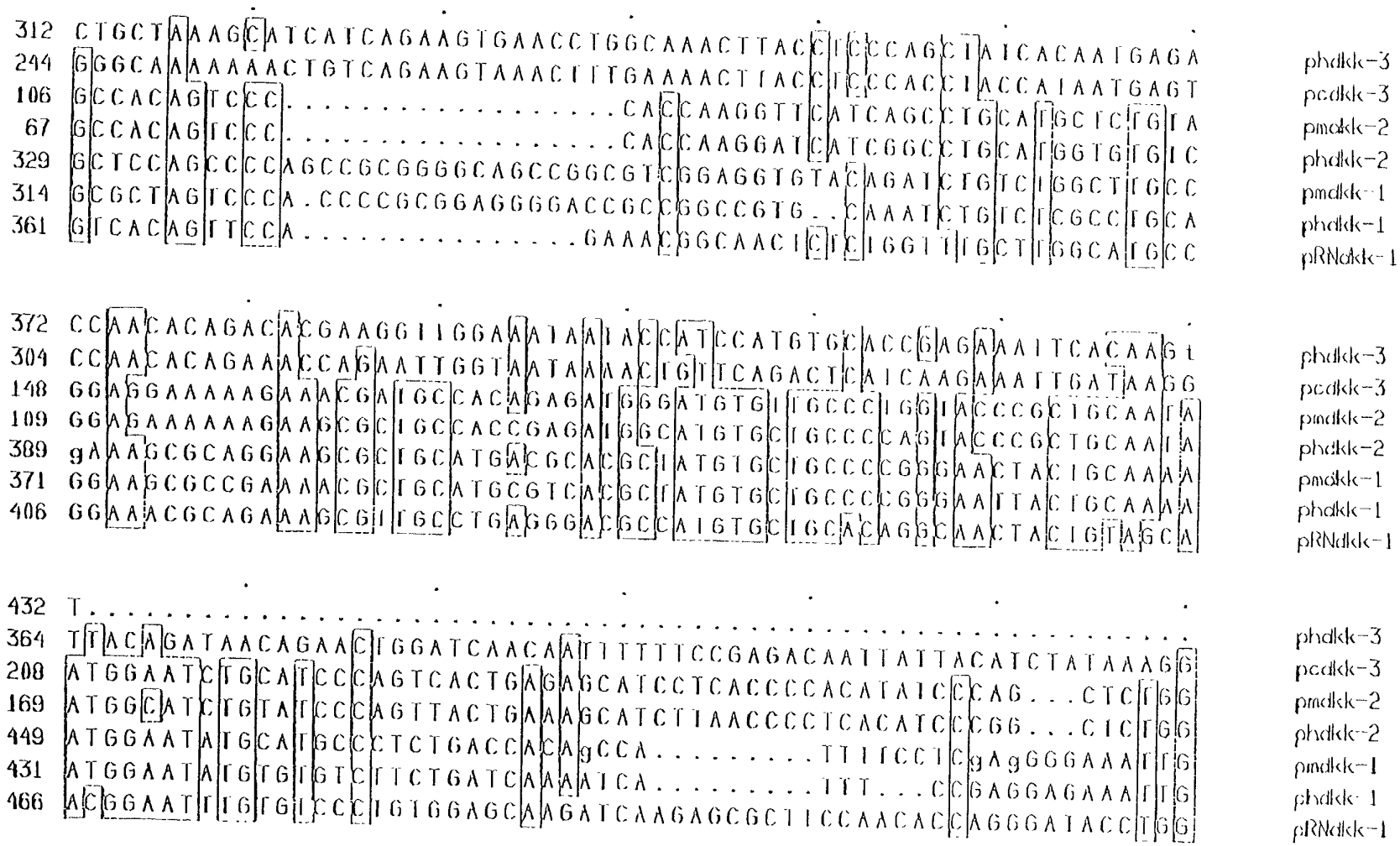
133 CACGGCCCCCGCGCCCGCT.CCGACGGCGACCTCGGCTCCAGTCAAGCCCGGCCCGGCTC   phdkk-3
 64 GGCGGCGATGGCTGCTGCTGTTGGCCGTGCTGGCGGCTCTGTGCTGCGCCGCGGCCGGGA   pcdkk-3
  1 ............................................................   pmdkk-2
  1 ............................................................   phdkk-2
152 ATTCCAACGCGATCAAGAACCTGCCCCCACCGCTGGGTGGTGCTGGGGGGCAGCCGGG..   pmdkk-1
141 GGGAGCAGTGGGCACTAACCGGTTTTGGAGAGGTGGACACATAAgGACTGTGATCAGC..   phdkk-1
181 TGACCAACTCCAACTCCATCAAGAATGTGCCGGCGGCACCAGCAGGTCAGCCCATTGGCT   pRNdkk-1

192 TCAGCTACCCGCAGGAGGAGGCCACCCTCAATGAGATGTTCCGCGAGGTTGAGGAACTGA   phdkk-3
124 GCGGCGGGCGGCGGCGAGCGGCCAGCCTGGGCGAGATGCTGCGGGAGGTGGAGGCGCTGA   pcdkk-3
  1 ...............TGCAGGCATGAACAAGGACTGGGTTCGGCGGCAGTGAGAAGGGCA   pmdkk-2
  1 ......................................................ATACGA   phdkk-2
210 .CTCTGCTGTCAGTGTGGCGCCGGGAGTTCTCTATGAGGGCGGGAACAAGTACCAGACTC   pmdkk-1
199 .GCCCGGGTCCAagAGGGCGGGTACCTGgACCTCTGGGTGCCTCAcCCTCTCCCCGAACC   phdkk-1
241 ACTACCCTGTGAGCGTCAGTCCGGACTCCCTATATGATATTGCCAACAAGTACCAACCTC   pRNdkk-1

252 TGGAGGACACGCAGCACAAATTGCGCAGCGCGGTGGAAGAGATGGAGGCAGAAGAAGCTG   phdkk-3
184 TGGAGGACACGCAGCACAAGCTGCGCAACGCCGTGCAGGAGATGGAAGCTGAAGAAGAAG   pcdkk-3
 46 AAAGCCTGGGGCAGGCCTACCCTTGCAGCAGTGATAAGGAATGTGAAGTTGGAAGATACT   pmdkk-2
  7 CTCACTATAGGGAATTTGGCCCTCGAGGCCAAGAATTCGGCACGAGGGTTGGGAGGTATT   phdkk-2
269 TTGACAACTACCAGCCCTACCCTTGCGCTGAAgATGAGGAGTGCGGCTCTGACGAGTACT   pmdkk-1
258 CTTCCCA....CAGCCGTACCCGTGCGCAGAGGACGAGGAGTGCGGCACTGATGAGTACT   phdkk-1
301 TGGATGCCTACCCGCTCTACAGTTGCACGGAAGATGATGACTGTGCCCTTGATGAATTCT   pRNdkk-1
```

Fig. 2 (Forts.)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244 GGGCAAAAAAACTGTCAGAAGTAAACTTTGAAAACTTACCTCCCACCIACCAIAATGAGT
106 GCCACAGTCCC..................CACCAAGGTTCATCAGCCTGCATGGCTCTGTA
67 GCCACAGTCCC..................CACCAAGGATCATCGGCCTGCATGGTGTGIC
329 GCTCCAGCCCCAGCCGCGGGGCAGCCGGCGTCGGAGGTGTACAGATCTGTCTGGCTTGCC
314 GCGCTAGTCCCA.CCCCGCGGAGGGGACCGCCGGCCGTG..CAAATCTGTCTCGCCTGCA
361 GTCACAGTTCCA..............GAAACGGCAACTCTCTGGTTTGCTTGGCATGCC
phdkk-3
pcdkk-3
pmdkk-2
phdkk-2
pmdkk-1
phdkk-1
pRNdkk-1
372 CCAACACAGACACGAAGGTTGGAAATAATACCATCCATGTGCACCGAGAAATTCACAAGt
304 CCAACACAGAAACCAGAATTGGTAATAAAACTGTTCAGACTCAICAAGAAATTGATAAGG
148 GGAGGAAAAAAGAAACGAIGCCACAGAGATGGGATGTGTTGCCCTGGTACCCGCTGCAATA
109 GGAGAAAAAAAGAAGCGCTGCCACCGAGATGGCATGTGCTGCCCCAGTACCCGCTGCAATA
389 gAAAGCGCAGGAAGCGCTGCATGACGCACGCTATGTGCTGCCCCGGGAACTACTGCAAAAA
371 GGAAGCGCCCGAAAACGCTGCATGCGTCACGCTATGTGCTGCCCCGGGAATTACTGCAAAAA
406 GGAAACGCAGAAAGCGTTGCCTGAGGGACGCCAIGTGCTGCACAGGCAACTACTGTAGCAA
phdkk-3
pcdkk-3
pmdkk-2
phdkk-2
pmdkk-1
phdkk-1
pRNdkk-1
432 T.....
364 TTACAGATAACAGAACTGGATCAACAATTTTTTCCGAGACAATTATTACATCTATAAAGG
208 ATGGAATCTGCATCCCAGTCACTGAGAGCATCCTCACCCCACATAICCCAG...CTCTGG
169 ATGGCATCTGTATCCCAGTTACTGAAAGCATCTTAACCCCTCACATCCCGG...CICTGG
449 ATGGAATATGCATGCCCTCTGACCACAgCCA.........TTTTCCTCgAgGGGAAATTTG
431 ATGGAATATGTGTGTCTTCTGATCAAAATCA.........TTT...CCGAGGAGAAATTTG
466 ACGGAATTTGTGTCCCIGTGGAGCAAGATCAAGAGCGCTTCCAACACCAGGGATACCTGG
phdkk-3
pcdkk-3
pmdkk-2
phdkk-2
pmdkk-1
phdkk-1
pRNdkk-1

Fig. 2 (Forts.)

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433 ............................................................ phdkk-3
424 GTGGAGAAAACAAAAGAAATCATGAGTGTATCATTGATGAAGACTGTGAAACAGGAAAGT pcdkk-3
265 ATGGCACCCGGCATAGAGATCGCAACCATGGTCACTATTCCAACCATGACCTGGGATGGC pmdkk-2
226 ATGGTACTCGGCACAGAGATCGAAACCACGGTCATTACTCAAACCATGACTTGGGATGGC phdkk-2
500 AgGAAAGCATCATTgAaAACCTTGGTAATGACCACaACgCCGCCGCGGGGATGGATATC pmdkk-1
479 AGGAAACCATCACTGAAAGCTTTGGTAATGATCATAGCACC......TTGGATGGGTATT phdkk-1
526 AAGAAACCATTCTGGAAAACTATAATAATGCTGATCATGC...AACAATGGATACTCATT pRNdkk-1

433 ............................................................ phdkk-3
484 ATTGCCAGTTCTCCACCTTTGAATACAAGTGTCAGCCCTGTAAAACCCAGCATACACACT pcdkk-3
325 AGAATCTAGGAAGGCCACACTCCAAGATGCCTCATATAAAAGGACATGAAGGAGACCCAT pmdkk-2
286 AGAATCTAGGAAGACCACACACTAAGATGTCACATATAAAAGGGCATGAAGGAGACCCCT phdkk-2
560 CCAgAAGAACcACACTGAcTtCAAAAaTATATCaCACCaaaGGACAaGAAGGCTCCGTCT pmdkk-1
533 CCAGAAGAACCACCTTGTCTTCAAAAATGTATCACACCAAAGGACAAGAAGGTTCTGTTT phdkk-1
583 CCAAATTAACCACGTCCCCATCTGGAATGCAGCCCTTTAAAGGCCGTGATGGTGATGTTT pRNdkk-1

433 ............................................................ phdkk-3
544 GCTCACGAGATGTTGAATGCTGCGGAGACCAGCTTTGTGTTTGGGGTGAGTGCAGGAAAG pcdkk-3
385 GCCTACGGTCATCAGACTGCATTGATGGGTTTTGTTGTGCTCGCCACTTCTGGACCAAAA pmdkk-2
346 GCCTACGATCATCAGACTGCATTGAAGGGTTTTGCTGTGCTCGTCATTTCTGGACCAAAA phdkk-2
620 GCCTCCgATCATCAgACTGTgCCgCagGGCTGTgTTGTGCaAgAcACTTCTGGTCCAAGA pmdkk-1
593 GTCTCCGGTCATCAGACTGTGCCTCAGGATTGTGTTGTGCTAGACACTTCTGGTCCAAGA phdkk-1
643 GCCTCCGATCAACTGACTGTGCGCCAGGTCTATGCTGTGCCCGTCATTTCTGGTCAAAGA pRNdkk-1
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Fig. 2 (Forts.)

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433 ............................................................  phdkk-3
604 CCACTTCAAGAGGAGAAAATG.GTACCATTTGTGAGAACCAACATGACTGCAACCCAGGA  pcdkk-3
445 TCTGCAAACCAGTGCTCCATCAGGGGGAAGTCTGTACCAAACAACGCAAGAAGGGTTCGC  pmdkk-2
406 TCTGCAAACCAGTGCTCCATCAGGGGGAAGTCTGTACCAAACAACGCAAGAAGGGTTCTC  phdkk-2
680 TCtgtAAACCTGTCCttaAAgAAGGTCaGGTGTGCACCAAGCACAAACGGAAAGGCTCCC  pmdkk-1
653 TCTGTAAACCTGTCCTGAAAGAAGGTCAAGTGTGTACCAAGCATAGGAGAAAAGGCTCTC  phdkk-1
703 TCTGCAAGCCGGTCCTTGATGAAGGCCAAGTGTGCACCAAGCACAGGAGGAAAGGCTCTC  pRNdkk-1

433 ............................................................  phdkk-3
663 ACGTGCTGTGCTTTTCAGAAAGAACTGCTGTTTCCTGTGTGCACTCCGTTACCCGAAGAA  pcdkk-3
505 ACGGGCTGGAGATTTTCCAGAGGTGTGACTGTGCAAAGGGCCTGTCCTGCAAAGTGTGGA  pmdkk-2
466 ATGGGCTGGAAATTTTCCAGCGTTGCGACTGTGCGAAGGGCCTGTCTTGCAAAGTATGGA  phdkk-2
740 ACGGGCTGGAgATATTCCAGCGCTGTTACTGCGGGGAAGGCCTGGCTTGCAGGATACAGA  pmdkk-1
713 ATGGACTAGAAATATTCCAGCGTTGTTACTGTGGAGAAGGTCTGTCTTGCCGGATACAGA  phdkk-1
763 ACGGGCTAGAGATTTTCCAGCGTTGTCACTGCGGTGCCGGACTCTCGTGCCGGTTACAGA  pRNdkk-1

433 ............................................................  phdkk-3
723 GGTGAACCTTGCCATGATCCTTCAAACAGACTTCTCAACCTGATCACCTGGGAACTGGAA  pcdkk-3
565 AAGATGCCACCTACTCTTCCA......AAGCCAGACTCCATGTATGCCAGAAGATCTGAT  pmdkk-2
526 AAGATGCCACCTACTCCTCCA......AAGCCAGACTCCATGTGTGTCAGAAAATTTGAT  phdkk-2
800 AAGATCACCATCAA...GCCAGCAATTCTTCTAGGCTCCACACCTGCCAGAGACACTAA.  pmdkk-1
773 AAGATCACCATCAA...GCCAGTAATTCTTCTAGGCTTCACACTTGTCAGAGACACTAA.  phdkk-1
823 AAGGAGAATTTACAACTGTCCCTAAAACATCGAGACTTCACACTTGCCAAAGACACTAAG  pRNdkk-1
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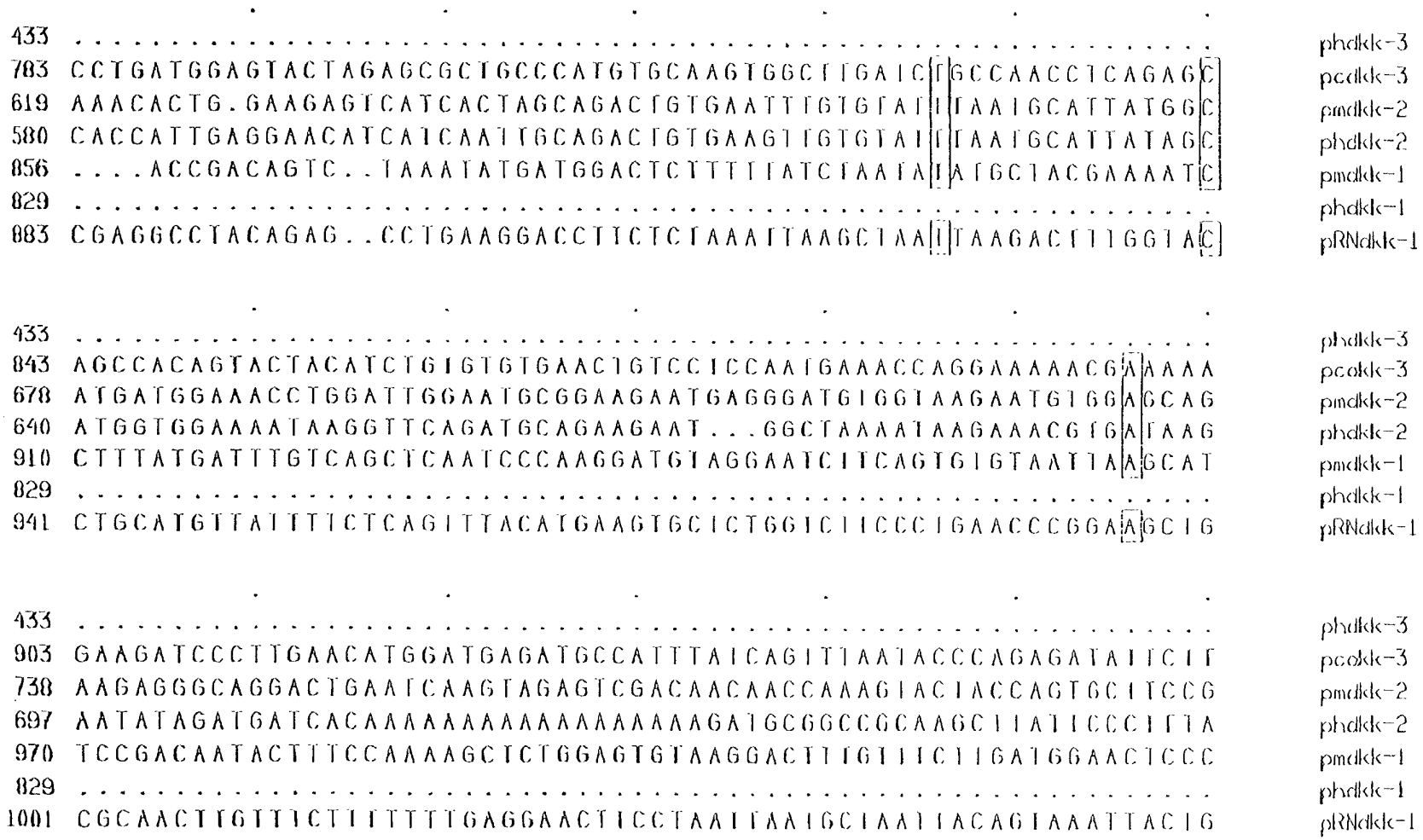
Fig. 2 (Forts.)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619 pmdkk-2
580 phdkk-2
856 pmdkk-1
829 phdkk-1
883 pRNdkk-1
433 phdkk-3
843 pcokk-3
678 pmdkk-2
640 phdkk-2
910 pmdkk-1
829 phdkk-1
941 pRNdkk-1
433 phdkk-3
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Fig. 2 (Forts.)

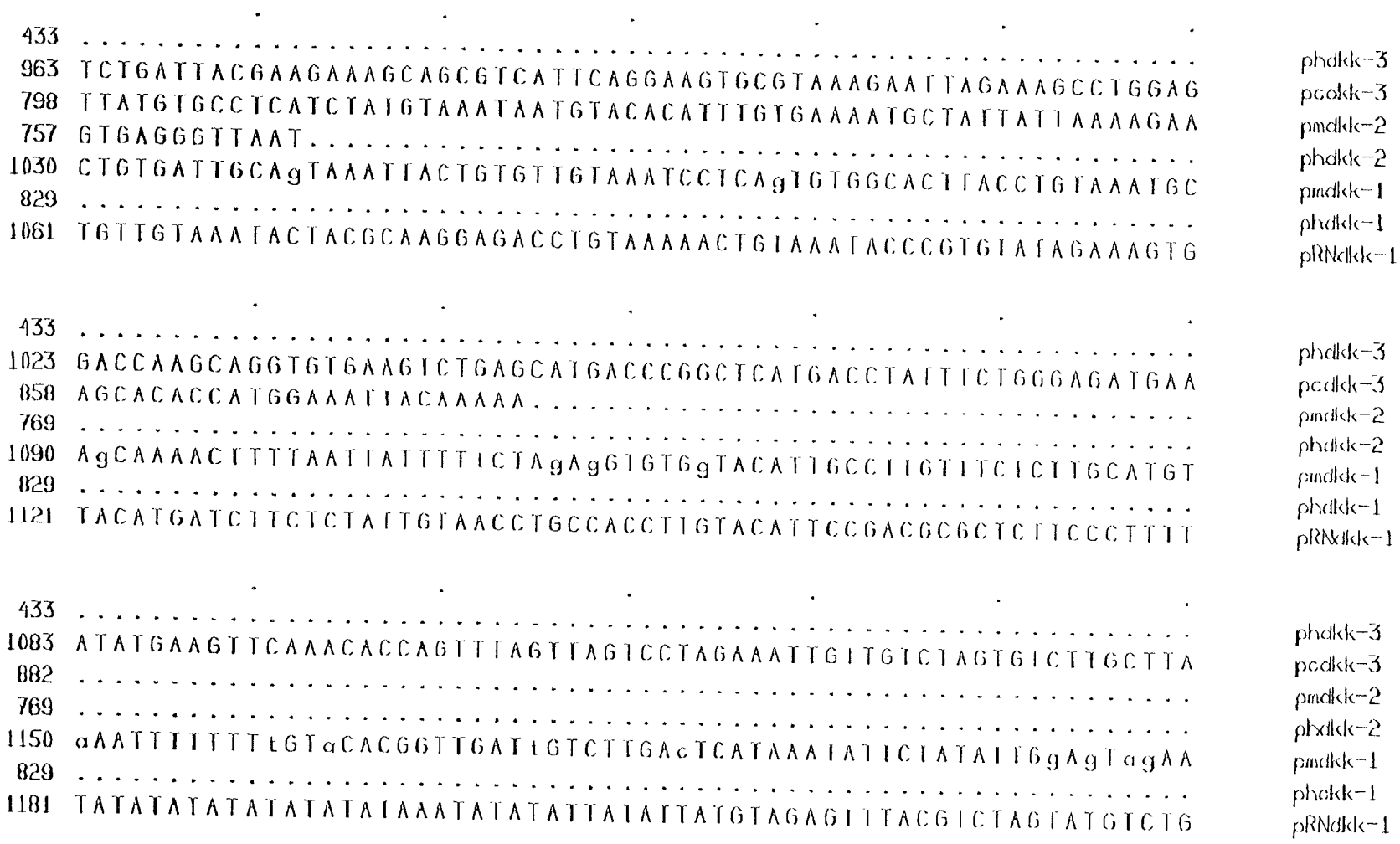
433 ..... phdkk-3
963 TCTGATTACGAAGAAAGCAGCGTCATTCAGGAAGTGCGTAAAGAATTAGAAAGCCTGGAG pcokk-3
798 TTATGTGCCTCATCTATGTAAATAATGTACACATTTGTGAAAATGCTATTATTAAAAGAA pmdkk-2
757 GTGAGGGTTAAT..... phdkk-2
1030 CTGTGATTGCAgTAAATTACTGTGTTGTAAATCCTCAgTGTGGCACTTACCTGTAAATGC pmdkk-1
829 ..... phdkk-1
1061 TGTTGTAAATACTACGCAAGGAGACCTGTAAAAACTGTAAATACCCGTGTATAGAAAGTG pRNdkk-1
433 ..... phdkk-3
1023 GACCAAGCAGGTGTGAAGTCTGAGCATGACCCGGCTCATGACCTATTTCTGGGAGATGAA pcdkk-3
858 AGCACACCATGGAAATTACAAAAA..... pmdkk-2
769 ..... phdkk-2
1090 AgCAAAACTTTTAATTATTTTTCTAgAgGTGTGgTACATTGCCTTGTTTCTCTTGCATGT pmdkk-1
829 ..... phdkk-1
1121 TACATGATCTTCTCTATTGTAACCTGCCACCTTGTACATTCCGACGCGCTCTTCCCTTTT pRNdkk-1
433 ..... phdkk-3
1083 ATATGAAGTTCAAACACCAGTTTAGTTAGTCCTAGAAATTGTTGTCTAGTGTCTTGCTTA pcdkk-3
882 ..... pmdkk-2
769 ..... phdkk-2
1150 aAATTTTTTTTtGTaCACGGTTGATtGTCTTGAcTCATAAATATTCTATATTGgAgTagAA pmdkk-1
829 ..... phdkk-1
1181 TATATATATATATATATAAATATATATTATATTATGTAGAGTTTACGTCTAGTATGTCTG pRNdkk-1

Fig. 2 (Forts.)

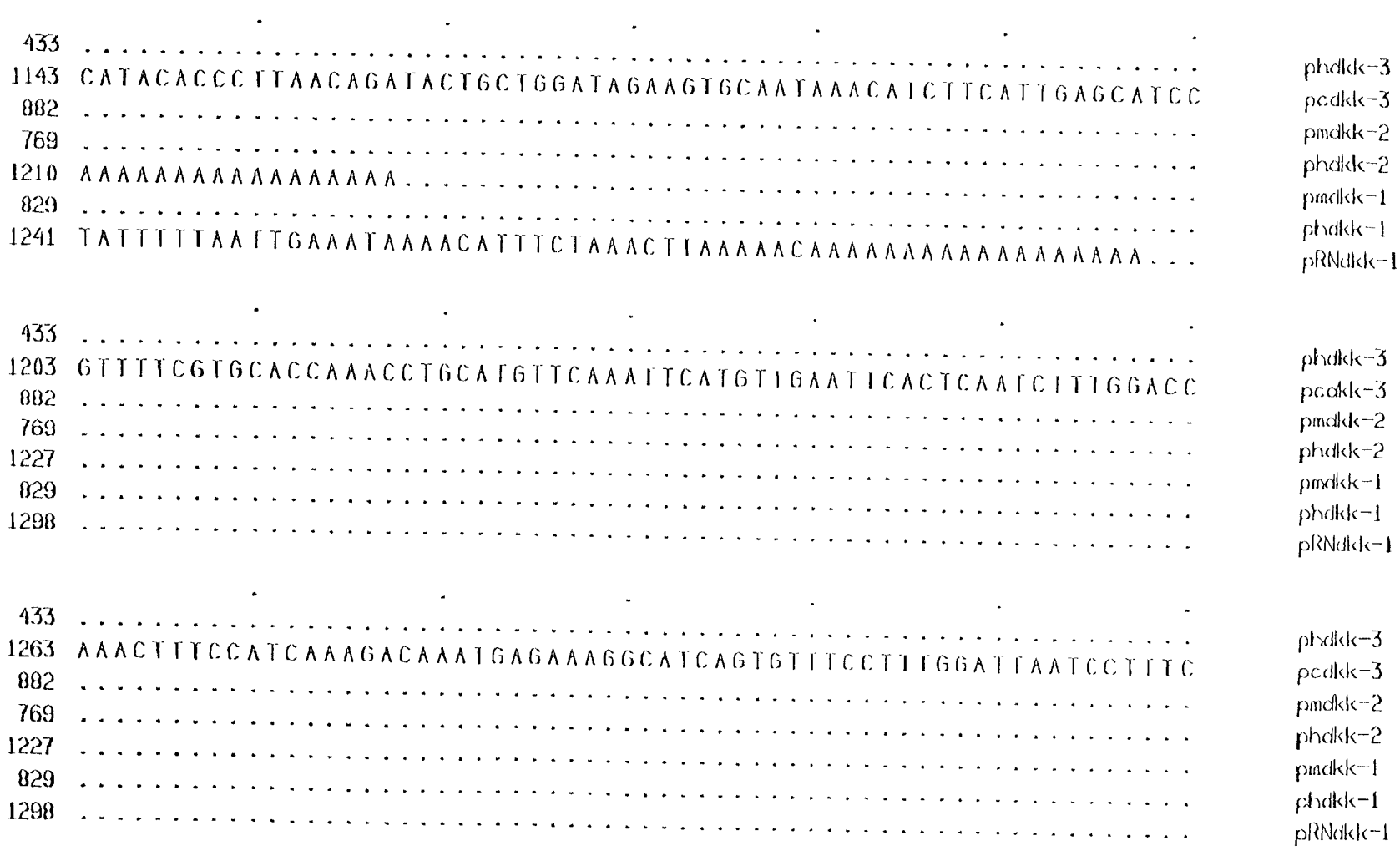
433 . . . . . phdkk-3
1143 CATACACCCTTAACAGATACTGCTGGATAGAAGTGCAATAAACATCTTCATTGAGCATCC pcdkk-3
882 . . . . . pmdkk-2
769 . . . . . phdkk-2
1210 AAAAAAAAAAAAAAAAA. pmdkk-1
829 . . . . . phdkk-1
1241 TATTTTTAATTGAAATAAAACATTTCTAAACTTAAAAACAAAAAAAAAAAAAAAAAA. . . pRNdkk-1
433 . . . . . phdkk-3
1203 GTTTTCGTGCACCAAACCTGCATGTTCAAATTCATGTTGAATTCACTCAATCTTTGGACC pcdkk-3
882 . . . . . pmdkk-2
769 . . . . . phdkk-2
1227 . . . . . pmdkk-1
829 . . . . . phdkk-1
1298 . . . . . pRNdkk-1
433 . . . . . phdkk-3
1263 AAACTTTCCATCAAAGACAAATGAGAAAGGCATCAGTGTTTCCTTTGGATTAATCCTTTC pcdkk-3
882 . . . . . pmdkk-2
769 . . . . . phdkk-2
1227 . . . . . pmdkk-1
829 . . . . . phdkk-1
1298 . . . . . pRNdkk-1

Fig. 2 (Forts.)

```
433   ............................................................  phdkk-3
1323  CTTTGTACAGCAGAAATAAACGTATCAGTACTCGTACTCATTAAAAAAACACACGGAGCA  pcdkk-3
882   ............................................................  pmdkk-2
769   ............................................................  phdkk-2
1227  ............................................................  pmdkk-1
829   ............................................................  phdkk-1
1298  ............................................................  pRNdkk-1

433   .                                                             phdkk-3
1383  T                                                             pcdkk-3
882   .                                                             pmdkk-2
769   .                                                             phdkk-2
1227  .                                                             pmdkk-1
829   .                                                             phdkk-1
1298  .                                                             pRNdkk-1
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Fig. 2 (Forts.)

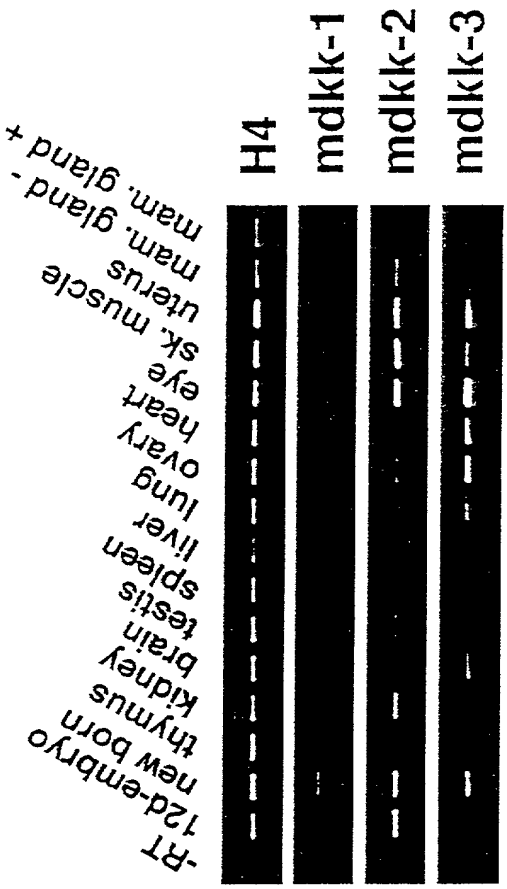
H4
mdkk-1
mdkk-2
mdkk-3
-RT
12d-embryo
new born
thymus
kidney
brain
testis
spleen
liver
lung
ovary
heart
eye
sk. muscle
uterus
mam. gland -
mam. gland +

Fig. 3

File No. 40168

DECLARATION AND POWER OF ATTORNEY

As below-named inventors, we hereby declare that our residences, post office addresses and citizenship are as stated below next to our names; we believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: INHIBITOR PROTEIN OF THE WNT SIGNAL PATHWAY, described in the specification filed in the U.S. Patent and Trademark Office on 27 April 2000 and assigned Serial No. 09/530,219.

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims.

We acknowledge the duty to disclose information which is material to the examination of the instant application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

We hereby claim priority benefits under Title 35, United States Code §119, §172 or §365 of any foreign application for patent or inventor's certificate, of any PCT international application designating at least one country other than the United States of American or of any provisional application listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application designating at least one country other than the United States of America filed by us on the same subject matter having a filing date before that of the application on which priority is claimed:

Prior Foreign Applications

Application No.	Country	Date Filed	Priority Claimed
PCT/DE98/03155	PCT	27 October 1998	Yes
197 47 418.7	DE	27 October 1997	Yes

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(None)		
(Application No.)	(Filing Date)	(Status: patented, pending, abandoned)

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

We hereby appoint David S. Abrams, Reg. No. 22,576; Robert H. Berdo, Reg. No. 19,415; Alfred N. Goodman, Reg. No. 26,458; Mark S. Bicks, Reg. No. 28,770; John E. Holmes, Reg. No. 29,392; Lance G.

Johnson, Reg. No. 32,531; Dean H. Nakamura, Reg. No. 33,981; and Garrett V. Davis, Reg. No. 32,023; Joseph J. Buczynski, Reg. 35,804; and Stacey J. Longanecker, Reg. 33,952 all of ~~ROYLANCE, ABRAMS, BERDO & GOODMAN, L.L.P., WHOSE ADDRESS IS 1300 19TH STREET, SUITE 600, WASHINGTON, DC 20036, TELEPHONE NUMBER (202) 659-9076~~, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

PLEASE DIRECT ALL CORRESPONDENCE AND TELEPHONE CALLS TO DEAN H. NAKAMURA AT THE ABOVE ADDRESS AND TELEPHONE NUMBER.

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